Interplay of atherogenic factors, protein intake and betatrophin levels in obese-metabolic syndrome patients treated with hypocaloric diets

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ABSTRACT

CONTEXT. The understanding of the potential role of betatrophin in human metabolic disorders is a current challenge.

OBJECTIVE. The present research evaluated circulating betatrophin levels in obese patients with metabolic syndrome features under energy-restricted weight-loss programs and in normal weight in order to stablish the putative interplay between the levels of this hormone, diet and metabolic risk factors linked to obesity and associated comorbidities.

SUBJECTS AND METHODS: One-hundred and forty three participants were enrolled in the study (95 obese-metabolic syndrome; age 49.5±9.4 y.o; BMI 35.7±4.5 kg/m2 / 48 normal-weight; age 35.71±8.8 y.o; BMI 22.9±2.2 kg/m^2). A nutritional therapy consisting in two hypocaloric strategies (Control diet based on the AHA recommendations and the RESMENA diet, a novel dietary program with changes in the macronutrient distribution) was only prescribed to obese-metabolic syndrome participants who were randomly allocated to the dietary strategies. Dietary records, anthropometrical and biochemical variables as well as betatrophin levels were analysed before (pre-intervention, wk 0), at 8 weeks (post-intervention, wk 8) and after 4 additional months of self-control period (follow-up, wk 24)

RESULTS. Betatrophin levels were higher in obese-metabolic syndrome patients than normal-weight subjects (1.24±0.43 ng/mL vs. 0.97±0.69 ng/mL, respectively, p=0.012), and levels were positively associated with body composition, metabolic parameters, leptin and irisin in all participants at baseline. Notably, low pre-intervention (wk0) betatrophin levels in obese patients were significantly associated with higher dietary-induced changes in atherogenic risk factors after 8 weeks. Moreover, protein intake, especially proteins from animal sources, was an independent determinant of betatrophin levels after dietary treatment (B=-0.27; p=0.012).

CONCLUSIONS. Betatrophin is elevated in obese patients with metabolic syndrome features and is associated with poorer nutritional outcomes of adiposity and dyslipidemia traits after a weight-loss program. Dietary protein intake could be a relevant modulator of betatrophin secretion and activity.

INTRODUCTION

Obesity is a worldwide health problem, and it is a worldwide epidemic at present [1]. The prevalence of obesity is increasing rapidly in most countries, and it is a major driving force for the increased development of dyslipidemia and glucose intolerance [2]. These metabolic disorder commonly associated with an excess of adiposity consist of a cluster of features that are included in the metabolic syndrome (MetSyn) which often results in atherosclerosis, cardiovascular diseases, and diabetes [3].

The design of an integral treatment for patients with obesity/metabolic syndrome has been an elusive task until now. The contradiction of facing an universal epidemic without effective treatments likely reflects our lack of an adequate understanding of the foundations of obesity [4]. Greater insight into the mechanisms of energy and body weight homeostasis will translate into better knowledge for the treatment of the obese/metabolic syndrome patients, being important to investigate the underlying mechanism involved in the obesity comorbidities and at the same time to find interactions with the diet in order to offer a better holistic therapy for this syndrome [5].

The discovery of leptin secretion by the adipose tissue opened a new era in this quest for new insights in the basis of obesity [6]. The age of leptin primarily demonstrated the relevance of peripheral tissues, such as fat, in the regulation of metabolism and this view was endorsed by subsequent discoveries in other tissues such as gastric [7, 8] and distal intestine [9]. Considerable evidence emerged in the last several years, which suggests that muscle and muscle contraction during exercise may have regulatory activity on the overall metabolism by the secretion of other factors, such as irisin [10]. In this context, the findings of the role of betatrophin, which promotes pancreatic B-cell expansion and insulin secretion and improves glucose tolerance in mice [11], and the findings of three other independent groups that characterized this

79 novel nutritionally regulated factor that is secreted by liver and adipose tissue [11-17]
80 created high expectations to our understanding and ability to counteract dyslipidemia
81 and hyperglycemia. The emerging importance of betatrophin as a critical regulator of
82 metabolic pathways in preclinical models prompted rapid studies in humans to evaluate
83 the potential translation of findings in mice to the clinic. However, a relevant
84 controversy soon evidenced because some authors reported increased circulating levels
85 of betatrophin in type 2 diabetes and obesity (T2DM) [12, 18-22], but other authors
86 found no differences [23, 24] or a decrease in circulating levels of betatrophin under
87 these metabolic impairments [25]. These results challenged the potential role of
88 betatrophin as a therapeutic target in metabolic disorders. Further studies of variations
89 in circulating levels of betatrophin under a therapeutic weight loss program have not
90 been elucidated previously, and these results could shed light on these controversial
91 outcomes.

92 The aim of present study was to evaluate circulating betatrophin levels in obese
93 patients with metabolic syndrome features under energy-restricted weight-loss programs
94 and in normal weight in order to establish interplay between the levels of this hormone,
95 diet and metabolic risk factors linked to obesity and associated comorbidities. Thus, it
96 was examined (1) the differences in betatrophin levels between normal weight and
97 obese patients and its association with metabolic risk factors; (2) the association
98 between baseline betatrophin levels and the response to an energy restriction treatment
99 in obese patients on body composition and glucose and lipid metabolic factors; (3) the
100 time-course of betatrophin levels after a dietary treatment with different protein content
101 on betatrophin levels.
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103

SUBJECTS AND METHODS

Subjects

A total of 143 subjects were enrolled in this study. A group of 95 obese patients (50 men, 44 women), 49.5±9.4 years old with a body mass index (BMI) of 35.7±4.5 kg/m^2 who were interested in following a weight-loss program were recruited to participate in the study through local advertisements. Forty-eight normal-weight subjects (16 men, 32 women), 35.71±8.8 years old with a BMI of 22.9±2.2 kg/m^2 were enrolled as controls. All participants provided a medical history, physical examination, and routine laboratory tests. Normal-weight patients (BMI≤25 kg/m^2) reported no history of diabetes mellitus, high blood pressure (BP), or dyslipidemia and were healthy overall. Obese patients (BMI>25 kg/m^2) presented at least two of the International Diabetes Federation (IDF) criteria for metabolic syndrome (MetSyn) [26]. Some of the metabolic syndrome patients (23 %) included in this study were diagnosed for T2DM and they were on antidiabetic medication at the baseline visit. Moreover, 62% of metabolic syndrome patients showed insulin resistance according to the cutoff point of an HOMA>2.5. Exclusion criteria for study enrollment were pregnancy, alcohol or drug abuse and chronic prescriptions of other medical drugs different of antidiabetic medication.

Written informed consent to participate in the trial was obtained before the start of the study in agreement with the Helsinki Declaration and followed national and European Union guidelines. The protocol for this study was approved by the Clinica Universidad de Navarra Ethics Committee (065/2009) and appropriately registered al Clinical Trials.gov (NCT01087086).

Nutritional therapy program for weight loss

Obese patients followed a therapy program based on a nutritional intervention that was controlled by trained dieticians from the Department of Nutrition, Food Sciences and Physiology of the University of Navarra. Participants followed a therapy program based on the RESMENA-S (MEtabolic Syndrome REduction in Navarra) study, which was a randomized controlled intervention trial to improve clinical criteria and biomarkers associated with MetSyn using a dietary strategy for weight loss [27, 28]. The six-month study was performed in two sequential periods: one eight-week intervention period (intervention period) in which subjects received nutritional assessments every 15 days and a 4-month self-control period in which subjects followed the habits learned in the first period (Follow-up period). Two energy-restricted diets were prescribed and compared. A 30% energy restriction was applied to the total energy requirements of each patient. The Control diet was based on AHA guidelines and included 3-5 meals/day and a macronutrient distribution of 50-55% total caloric value from carbohydrates, 15% from proteins and 30% from lipids. The RESMENA diet was composed of 7 meals/day with a 40% total caloric value from carbohydrates, 30% from proteins and 30% from lipids. Anthropometrical, biochemical, and dietary intake variables were assessed at baseline (wk 0), after the intervention period (wk 8) and at the end of the study (wk 24). Dietary compliance was obtained through 48-hour weighed food records [27, 28], and diet composition was analyzed using the DIAL software (Alce Ingenieria, Madrid, Spain).

Anthropometrical and body composition measurements

Body weight measurements were performed using a digital balance that was accurate to 0.1 kg (Seca 767; Vogel & Halke, Germany), and height was measured

using a wall-mounted stadiometer (Seca 220; Vogel &Halke). Waist circumference was measured at the site of the smallest circumference between the rib cage and the iliac crest. Body composition was measured using bioelectrical impedance (TANITA sc-330; Tanita corporation). All measurements were obtained with the subjects in underwear and after an overnight fast. Anthropometric and body composition measurements were performed according to previously described standardized procedures [29].

Hormonal and biochemical analyses

Venous blood samples were drawn after a 12-h overnight fast at the beginning of the restriction diet (pre-intervention, wk 0), the end of the diet intervention (post-intervention, wk 8), and 4 months after the self-control period (follow-up, wk 24). Blood samples from normal-weight subjects were obtained under basal conditions after an overnight fast. EDTA-plasma and serum of specimens were separated from whole blood and immediately frozen at -80º C until assay.

The quantitative measurement of betatrophin in human plasma samples was analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals, Inc., CA), according to the manufacturer's instructions. Absorbance from each sample was measured in duplicate using a spectrophotometric microplate reader at a wavelength of 450 nm (Versamax Microplate Reader; Associates of Cape Cod Incorporated, East Falmouth, MA).

Serum concentrations of glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), homocysteine, free fatty acids (FFA), aspartate aminotransferase (AST) and aspartate aminotransferase (AST) were measured using an autoanalyzer (Pentra C-200; HORIBA ABX, Madrid, Spain) with specific kits. Low-density lipoprotein-cholesterol (LDL-c) levels were calculated using the

Friedewald formula [30]. Apolipoproteins AI and B were measured with specific kits (Tina-quant Apolipoprotein A-I ver.2 and Tina-quant Apolipoprotein B ver.2) with the help of an autoanalyzer (Roche/Hitachi model 904/911/912/917/ Modular: Roche Diagnostics).

Overnight fasting plasma levels of ghrelin, leptin and adiponectin were measured using commercially available ELISA kits (Millipore, MA, USA). Plasma levels of irisin were measured using a commercial ELISA kit that was directed against amino acids 31-143, which includes the extracellular part of the FNDC5 protein (Irisin ELISA kit EK-067-52; Phoenix Pharmaceuticals, CA). Insulin concentrations were assessed using an ELISA kit from Mercodia, AB (Uppsala, Sweden). Insulin resistance was indirectly determined using the homeostatic model assessment index (HOMA-IR), which was calculated as follows: [fasting plasma glucose (mM) x fasting plasma insulin (μU/mL)/22.5], as described previously [31].

Statistical analysis

The sample size of the current trial was calculated to detect differences for betatrophin between obese and normal-weight patients taking into account published values of circulating betatrophin standard deviation [12]. It was calculated for an effect size ≥0.5 ng/mL, and α=0.05, and a power (1-β) of 80%. Thus, the sample size was established at a minimum of 15 volunteers per group to detect differences according to the adiposity levels. The sample size provided sufficient power to test for effects on a number of other metabolic variables of interest.

The normal distribution of variables was explored using the Kolmogorov-Smirnov and Shapiro-Wilk tests. An ANCOVA was used to study differences between groups adjusted for gender and age. A repeated-measures ANCOVA was used to study

the effects of time of the nutritional therapy program and groupings on body weight and biochemical parameters in obese patients adjusted for gender. The potential association between anthropometrical and biochemical parameters and betatrophin levels were evaluated using the Spearman coefficient test.

The effects of high or low pre-intervention betatrophin levels on dietary-induced changes and the association between protein intake and post-treatment betatrophin levels were analyzed using the median (above and below the 50^{th} percentile) cutoff values of betatrophin as previously applied [32] and based on a validated method to assign the studied population into two groups of disease risk [33].

Multivariate linear regression models were fitted to assess the potential predictive factors of betatrophin levels after treatment and adjusted for age and gender. Three regression models were performed. Model 1 included variables for body composition and glucose and lipid metabolism-related parameters. Model 2 also included total protein and model 3 included animal protein intake.

Statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., Chicago, IL) for Windows XP (Microsoft, Redmond, WA). $P \leq 0.05$ was considered statistically significant.

RESULTS

Circulating levels of betatrophin were evaluated in 143 subjects, including 48 normal-weight and 95 metabolic syndrome patients of both genders, whose characteristics are shown in Supplementary Table 1. Circulating betatrophin concentrations were significantly higher in obese-metabolic syndrome subjects (1.24±0.06 ng/ml) than normal-weight subjects (0.97±0.08 ng/ml), and the difference was statistically significant (p=0.012) after adjusting for age and gender (Figure 1). Additionally,

229 although slightly low levels of betatrophin were observed in patients diagnosed with
230 T2DM, the difference was not statistically significant (1.27±0.051 ng/ml vs. 1.13±0.08;
231 p=0.180), neither when patients were classified according to the IDF criteria (1.25±0.08
232 vs. 1.23±0.05; p=0.873) nor regarding the insulin resistance (1.30±0.08 vs 1.20±0.05;
233 p=0.284) respect to the patients without these conditions. No gender-dependent
234 component was detected when 66 men (1.18±0.08 ng/ml) and 76 women (1.10±0.06
235 ng/ml) were compared (p=0.425), and no statistically significant interaction between
236 metabolic syndrome and gender (p=0.182) was observed (Figure 1).
237 Notably, circulating betatrophin levels in metabolic syndrome and normal-weight
238 patients together were associated with several anthropometric and body composition
239 measurements and biochemical variables related to lipid and glucose homeostasis
240 (Table 1). Betatrophin positively correlated with body weight (r=0.24; p=0.004), BMI
241 (r=0.30; p<0.001), waist circumference (r=0.23; p=0.014), fat mass (r=0.20; p=0.028)
242 and fat-free mass (r=0.23; p=0.013) measured in kg. Betatrophin levels also directly
243 associated with circulating levels of glucose (r=0.32; p<0.001), insulin (r=0.27;
244 p=0.001), HOMA (r=0.28; p=0.001) and triglycerides (r=0.18; p=0.028). Leptin
245 (r=0.24; p=0.012) and irisin (r=0.33; p<0.001) levels directly correlated with
246 betatrophin.
247 In the obese-metabolic syndrome patients, the nutritional treatment induced a
248 statistically significant weight loss over 8 weeks (-6.73±0.71 kg and -7.09± 0.82 kg in
249 the AHA and RESMENA groups, respectively) and a reduction in BMI, waist
250 circumference, body fat mass and biochemical variables without differences between
251 the dietary groups. Therefore, the obese-metabolic syndrome patients were merged and
252 distributed into two groups according to the median pre-intervention values of
253 betatrophin (1.21 ng/ml). Patients with pre-intervention (wk 0) betatrophin levels higher

than 1.21 ng/ml exhibited a lower decrease in fat mass percent (-2.36±0.45% vs. -3.38±0.30%; p=0.024) and greater diminution in fat-free mass (-2.09±0.43 kg vs. -1.03±0.30 kg; p=0.049) than patients with pre-intervention betatrophin levels lower than 1.21 ng/ml (Table 2). Notably, the diet-induced diminution in HDL was more severe in patients with pre-intervention betatrophin levels that were higher than the median. This finding was also evidenced in the atherogenic index TG/HDL (Table 2). In fact, pre-intervention betatrophin levels were associated with diet-induced changes in body fat mass (r=0.23; p=0.024), HDL levels (r=-0.33; p=0.001) and the TG/HDL index (r=0.25, p=0.016; Figure 2A-C), which indicates that greatest diet-induced benefit on atherogenic risk factors was achieved in patients with the lowest circulating betatrophin levels. No association was found between pre-intervention betatrophin levels and changes in glucose homeostasis factors, such as glucose, insulin and HOMA (Table 2).

Evaluations of the circulating betatrophin levels in all patients together following the nutritional treatment (Figure 3A) revealed no statistically significant differences (p=0.208) after the energy restriction (wk0: 1.25±0.05 ng/ml; wk8: 1.19±0.053 ng/ml) and subsequent follow-up (wk24: 1.25±0.053) period. However, the AHA group exhibited higher betatrophin levels than the RESMENA group after the intervention period (wk8) and follow-up period (wk24) (Figure 3A). These differences were statistically significant ($p<0.05$; Figure 3A). When the interaction diet x baseline betatrophin levels was examined in post-intervention betarophin levels, this interaction was not statistically significant (diet x baseline betatrophin levels interaction p-value=0.979). These results reveal an effect of the composition of the diet rather than the energy-restriction.

Then, the associations between betatrophin levels and protein intake after treatment were evaluated because the relevant difference between the dietary regimens included a

higher total protein intake in the RESMENA group [28]. Accordingly, patients who consumed a total protein intake below the median value (67.1 g/day) exhibited statistically significant higher betatrophin levels than patients who reported a daily total protein intake higher than 67.1 g/d after the intervention and follow-up period (Figure 3B). No statistically significant differences were detected in the daily intake of vegetable protein at wk8 and wk 24 (Figure 3C), but differences were observed for the daily intake of animal protein ($p<0.05$) (Figure 3D). Linear regression models revealed that 20% of the variability in post-treatment betatrophin levels was conjointly explained by the dietary-induced changes in body composition, lipid profile and animal-based protein intake within the hypocaloric diet (Table 3).

DISCUSSION

Metabolic syndrome prevalence has increased rapidly in the past two decades, which highlights the necessity for the development of effective therapeutic approaches [34]. Betatrophin, which is also named lipasin or ANGPTL8, is a protein that is encoded by the GM6484 gene in mice and C19orf80 in humans. Betatrophin is predominantly expressed in the human liver, and it is involved in the regulation of lipid metabolism. Betatrophin provides new hope for the treatment of hyperlipidemia, type 2 diabetes, obesity and related metabolic disorders [34].

The present study demonstrated high levels of ANGPTL8/betatrophin/lipasin in metabolic syndrome patients, and the circulating levels of this protein positively associated with anthropometric and body composition measures, circulating levels of glucose, insulin, HOMA-IR and triglycerides, and hormones, such as leptin and irisin. Notably, patients with low levels of pre-intervention betatrophin experienced better beneficial effects of the weight loss program on atherogenic, but not glucose

intolerance, risk factors than patients with high levels of betatrophin. Circulating levels of betatrophin did not vary following the energy-restricted weight-loss treatment in obese-metabolic syndrome patients despite the association between betatrophin and body composition measures. However, the dependence of betatrophin levels on total dietary protein intake, with circulating betatrophin levels being higher in patients with lower protein intake, was observed for the first time. This association was particularly evident after the intake of protein of animal origin but not after protein-derived from a vegetable source.

Betatrophin/lipasin-null mice exhibit reduced serum triglycerides [12, 16, 35], but betatrophin overexpression dramatically increases serum triglycerides [12, 17]. Moreover, a single-nucleotide polymorphism in the betatrophin gene, a mutation with a putative loss-of-function, is associated with an improved lipid profile in genome-wide association studies [14, 23]. Previous studies in humans revealed high levels of betatrophin in obesity and type 2 diabetes [12, 18-22] and a positive correlation between circulating betatrophin levels and serum lipid levels [23]. These observations are also consistent with our results in subjects with metabolic syndrome features. In contrast, a recent study concluded that serum betatrophin is decreased in human obesity, and this study reported an inverse association between betatrophin levels and obesity-associated cardiometabolic risk factors [25]. These differences may be due to methodological differences, including the use of different inmunoassays that detect different fragments (the N- and C-terminal kits) of betatrophin [36]. However, these discrepancies may also be due to disparities in the patients included in the studies. Our study included overweight and obese patients with metabolic syndrome and no statistical differences in betatrophin were observed in spite of high glucose profile and insulin resistance presence. Similar findings were recently reported [24]. These results and the data here

329 reported highlight the relevance of fat mass on the regulation of the betatrophin
330 circulating levels rather than the altered carbohydrate metabolism. Therefore, it could be
331 postulated that betatrophin is secreted as an adaptive response in overweight and
332 moderately obese metabolic syndrome patients to counteract the lipid and glucose
333 metabolism disorders that are associated with metabolic syndrome. This response of
334 betatrophin secretion could be dramatically disturbed and/or downregulated in high-risk
335 populations, such as morbid obese patients.

336 The findings observed in the current nutritional intervention study of metabolic
337 syndrome patients support this hypothesis. Patient categorization according to
338 betatrophin levels before the beginning of the nutritional treatment revealed that patients
339 with lower betatrophin levels exhibited better improvements in body fat mass and
340 atherogenic variables induced by the energy restriction treatment. This outcome
341 suggests that patients with low circulating betatrophin levels are in a more favorable
342 obesity-related state to achieve a greater effect of the energy restriction treatment in the
343 short-term than patients with high circulating betatrophin levels prior to therapeutic
344 intervention.

345 Importantly, the betatrophin levels did not vary following the energy restriction
346 treatment in spite of their association with body fat mass but differences were observed
347 depending on the assigned diet. The RESMENA diet in the current study differs from
348 the AHA diet because the former diet was designed to obtain 30% of energy from
349 proteins, and the AHA diet was designed to obtain only 15% of energy from proteins.
350 Patients following the RESMENA diet recorded higher protein intake than patients who
351 followed the AHA diet, and RESMENA patients exhibited a reduction in android fat
352 mass values, which is a marker of central obesity that is associated with increased risk
353 of metabolic syndrome manifestations [28], consistent with previous reports [37-39].

The current study demonstrated for the first time an inverse association between circulating betatrophin levels and protein intake within a hypocaloric diet. Paradoxically, this association was particularly marked with protein derived from animal sources because patients with higher animal protein intake showed lower betatrophin levels.

Protein derived from animal sources was associated with increased risks of global and abdominal obesity in healthy adults, which suggests that a lower animal protein intake is important for the maintenance of healthy body weight [40]. In contrast, vegetable protein intake appears to play a role in preventing obesity in European adolescents, which was observed in the HELENA cross-sectional study [41]. Moreover, a higher intake of animal protein, which was recorded in the RESMENA diet group [42] and the DiOGenes project [43], was associated with higher plasma levels of inflammatory markers in obese adults.

The RESMENA diet group also recorded higher antioxidant capacity (TAC) of the diet and a beneficial effect on oxidative stress markers than the AHA diet [28]. However, no association was observed in the current study between betatrophin levels and the TAC of the diet, oxidative stress, or circulating inflammatory markers (data not shown), which suggests that protein intake is the most relevant dietary factor in betatrophin regulation. In fact, the regression model evidenced that protein intake within an energy-restricted treatment, conjointly with the induced changes in body composition and lipid profile, determined betatrophin levels after the nutritional intervention (post-intervention).

The strength of this study is its longitudinal design which allows for the first time the evaluation of the time-course of changes of betathrophin as well as its association with the response to a weight-lowering treatment in obese patients. Meanwhile, because this

report describes an observational association study, this work is not able to demonstrate causality. The interplay between betatrophin levels and protein intake, quantity and quality, should be further explored in pre-clinical and clinical studies.

In conclusion, the current results demonstrate that betatrophin levels are increased in overweight and moderately obese patients with metabolic syndrome features and positively associated with adiposity, lipid metabolism and glucose homeostasis parameters. Notably, the current work demonstrates for the first time that lower baseline circulating betatrophin levels are associated with a higher efficacy of a dietary weight-reducing program in improving adiposity and dyslipidemia in obese patients with metabolic syndrome features. Moreover, the betatrophin concentration does not vary following the energy restriction treatment, but the levels are determined by the composition of the hypocaloric diet such as the content in animal-derived protein intake.

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404 Supplementary information is available at IJO's website.

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Figure legends

Figure 1. Comparison of circulating betatrophin levels according to adiposity. Betatrophin levels were higher in obese-metabolic syndrome patients than normal weight-healthy patients. The data are presented as the means (SE). *Significant differences ($p<0.05$) between normal-weight and metabolic syndrome patients evaluated using ANCOVA adjusted by age and gender.

Figure 2. Association between plasma betatrophin and diet-induced changes in percent body fat mass (A), circulating HDL levels (B), and the atherogenic index TG/HDL (C). HDL, high-density lipoprotein; TG, triglycerides.

Figure 3. (A) Plasma betatrophin levels during the nutritional treatment (weeks 0-8) and the 4-month follow-up period (week 24). Data are presented as the means (SE). *Statistically significant differences ($p<0.05$) between the AHA and RESMENA diet groups. (B-C) Comparison of betatrophin levels after energy restriction treatment with regard to the protein intake. * Significantly different ($p<0.05$) compared to low protein intake.

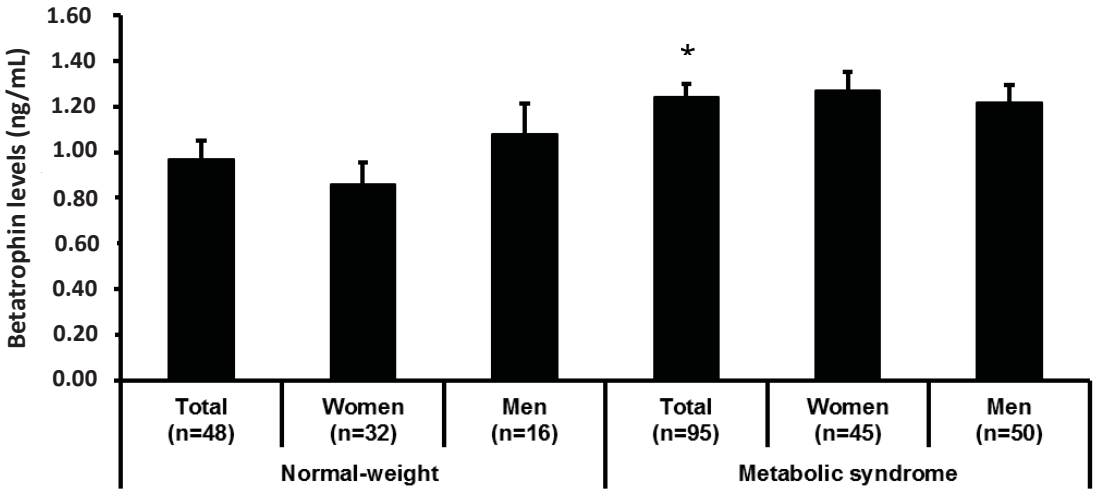

Betatrophin levels (ng/mL)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
*
Total (n=48)
Women (n=32)
Men (n=16)
Total (n=95)
Women (n=45)
Men (n=50)
Normal-weight
Metabolic syndrome

Figure 1

Table 1. Association between baseline betatrophin circulating levels and anthropometric and biochemical parameters in normal weight and metabolic syndrome patients (n=143)

	Spearman´s r coeficient	**p-value**
Age (years)	0.133	0.116
Body weigt (kg)	**0.244**	**0.004**
BMI (kg/m^2)	**0.303**	**<0.001**
Waist circumference (cm)	**0.227**	**0.014**
Fat mass (%)	-0.010	0.912
Fat mass (kg)	**0.200**	**0.028**
Fat-free mass (%)	-0.114	0.216
Fat-free mass (kg)	**0.226**	**0.013**
Glucose (mg/dL)	**0.316**	**0.000**
Insulin (mU/L)	**0.273**	**0.001**
HOMA-IR	**0.280**	**0.001**
Total Cholesterol (mg/dL)	0.045	0.599
HDL-c (mg/dL)	-0.071	0.408
Triglycerides (mg/dL)	**0.185**	**0.028**
Leptin (ng/mL)	**0.241**	**0.012**
Irisin (ng/mL)	**0.333**	**<0.001**

Table 2. Anthropometric and biochemical changes induced by the nutritional intervention according to pre-intervention (baseline) betatrophin circulating levels.

	Pre-intervention betatrophin lower levels (<1.21 ng/ml; n=47)					Difference		**Pre-intervention betatrophin higher levels (≥1.21 ng/ml; n=48)**					Difference		Difference *P* value
	Pre-intervention (wk0)		Post-intervention (wk8)		P value			Pre-intervention (wk0)		Post-intervention (wk8)		*P* value			
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD		Mean	SD	
Body weight (kg)	99.2	15.3	92.3	14.2	0.000	-6.8	2.9	99.9	20.1	92.9	19.7	0.000	-6.9	3.1	0.856
BMI (kg/m^2)	35.3	4.5	32.9	4.2	0.000	-2.4	1.0	36.3	4.8	33.7	4.9	0.000	-2.5	1.1	0.687
Waist circumference (cm)	110.5	12.4	103.6	11.1	0.000	-6.9	4.7	111.4	13.2	104.0	12.7	0.000	-7.4	4.2	0.576
Fat mass (%)	39.0	7.4	35.7	7.7	0.000	**-3.4**	2.1	39.3	6.7	36.9	7.1	0.000	**-2.4**	3.1	**0.024**
Free-fat mass (kg)	60.4	11.8	59.4	11.5	0.002	**-1.0**	2.1	60.6	13.8	58.5	13.3	0.000	**-2.1**	3.0	**0.049**
SBP (mmHg)	144.7	16.6	135.8	14.0	0.000	-8.9	14.8	153.1	20.5	138.0	18.7	0.000	-15.2	22.0	0.105
DBP (mmHg)	83.9	9.2	76.9	9.1	0.000	-7.0	7.4	85.5	9.1	79.0	10.0	0.000	-6.4	11.34	0.776
TC (mg/dL)	212.4	41.9	199.5	41.7	0.054	-12.9	44.2	225.9	43.3	208.2	43.1	0.001	-17.7	32.9	0.556
LDL-c (mg/D)	133.5	38.4	131.7	36.8	0.766	-1.8	40.9	141.6	37.9	133.7	37.6	0.036	-7.9	25.2	0.388
HDL-c (mg/dL)	42.6	9.3	41.5	8.8	0.281	**-1.0**	6.4	46.7	10.1	42.1	9.6	0.000	**-4.6**	7.2	**0.013**
TG/HDL-c (mg/dL)	0.58	0.27	0.47	0.23	0.000	**-0.11**	0.19	0.55	0.30	0.52	0.30	0.395	**-0.02**	0.17	**0.020**
TC/HDL-c (mg/dL)	5.1	1.2	4.9	1.1	0.195	-0.21	1.1	5.0	1.1	5.1	1.1	0.338	0.11	0.79	0.105
LDL/HDL-c (mg/dL)	3.2	0.8	3.2	0.91	0.729	0.05	1.0	3.1	0.75	3.2	0.80	0.127	0.15	0.66	0.555
ApoA1 (mg/dL)	129.0	18.4	119.6	18.2	0.000	-9.5	11.4	138.5	23.8	125.2	21.2	0.000	-13.2	16.2	0.199
ApoB (mg/dL)	90.8	22.4	81.4	20.2	0.000	-9.4	16.2	101.3	22.9	92.3	22.3	0.000	-9.0	13.6	0.878
Glucose (mg/dL)	123.3	38.2	108.7	21.5	0.009	-14.5	36.1	122.0	34.1	108.3	19.3	0.005	-13.6	31.6	0.894
Insulin (mU/L)	14.5	7.87	8.6	4.9	0.000	-5.9	5.3	14.9	11.6	9.6	7.8	0.000	-5.2	7.3	0.608
HOMA-IR	4.5	2.8	2.4	1.6	0.000	-2.1	1.9	4.6	3.9	2.7	2.5	0.000	-1.9	2.6	0.730
TG (mg/dL)	181.8	106.5	131.4	62.1	0.000	-50.4	82.0	187.6	112.0	162.0	102.7	0.012	-25.7	67.4	0.115
Homocisteine (µmol/L)	15.1	3.9	15.9	4.0	0.139	0.8	3.5	15.9	3.5	15.1	3.2	0.075	0.77	2.9	0.978
FFA (mg/dL)	0.53	0.19	0.53	0.26	0.929	0.00	0.26	0.56	0.21	0.48	0.19	0.011	-0.08	0.21	0.122
ALT (U/L)	32.1	14.1	29.9	13.4	0.320	-2.3	15.2	34.6	20.2	26.6	11.8	0.002	-8.1	16.9	0.084
AST (U/L)	23.9	8.9	22.3	6.1	0.200	-1.6	8.1	23.8	9.0	20.3	5.7	0.007	-3.5	8.6	0.256
Irisin (ng/mL)	345.7	201.9	266.3	122.7	0.000	-79.4	124.1	363.4	156.9	299.0	123.9	0.000	-64.4	81.2	0.491
Leptin (ng/mL)	20.4	17.6	12.4	11.7	0.000	-7.9	10.0	22.4	11.7	15.3	10.6	0.000	-7.1	8.6	0.669
Adiponectin (ng/mL)	10.8	7.9	14.5	17.8	0.191	3.7	18.8	15.1	11.0	17.2	17.5	0.418	2.1	17.6	0.676
Ghrelin (pg/mL)	431.5	265.7	526.5	318.8	0.003	95.1	163.4	371.7	117.4	418.4	142.4	0.024	46.6	116.4	0.167

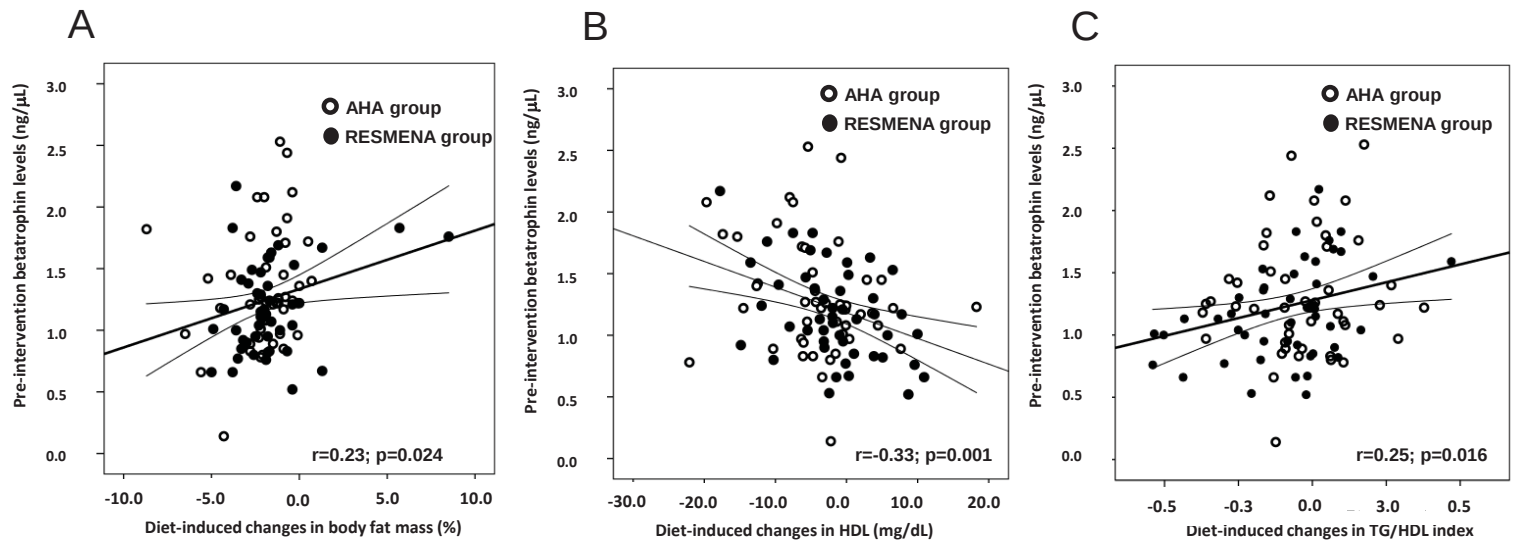
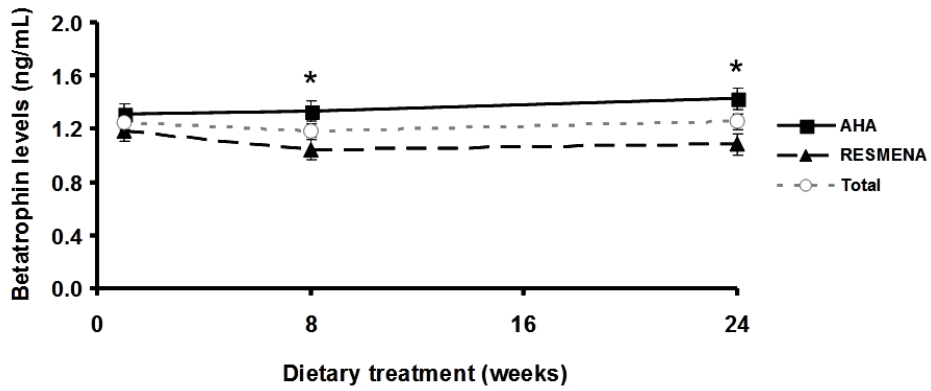

A
B
C
AHA group
RESMENA group
Pre-intervention betatrophin levels (ng/µL)
Diet-induced changes in body fat mass (%)
r=0.23; p=0.024
Diet-induced changes in HDL (mg/dL)
r=-0.33; p=0.001
Diet-induced changes in TG/HDL index
r=0.25; p=0.016

Figure2

A



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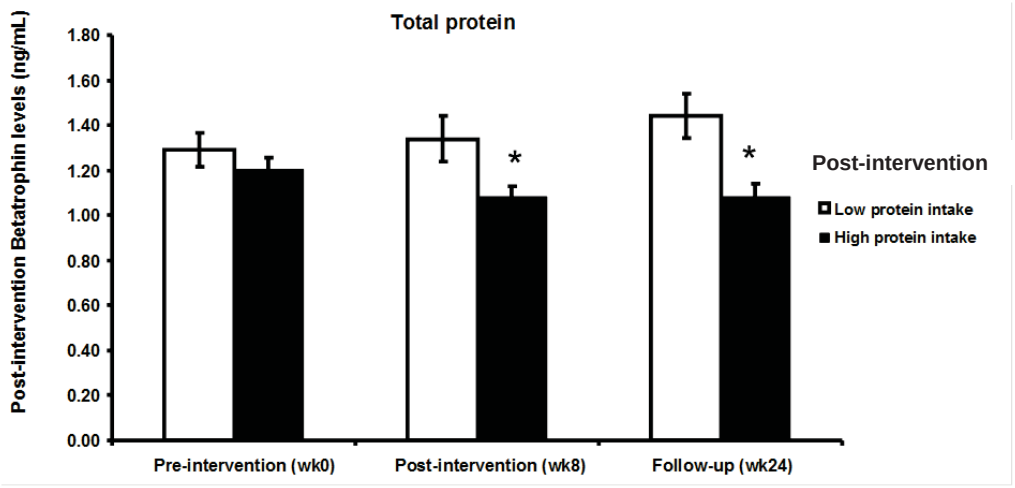

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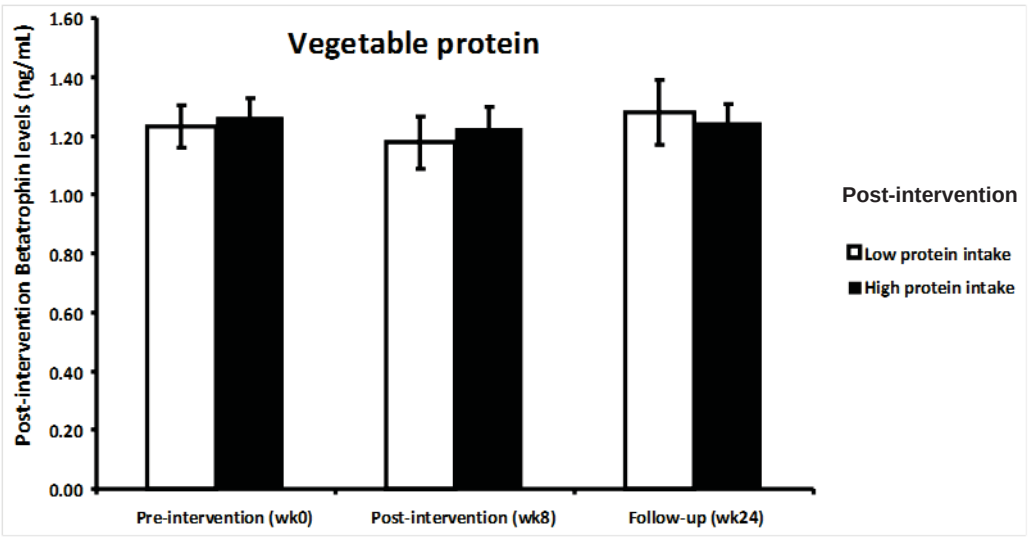

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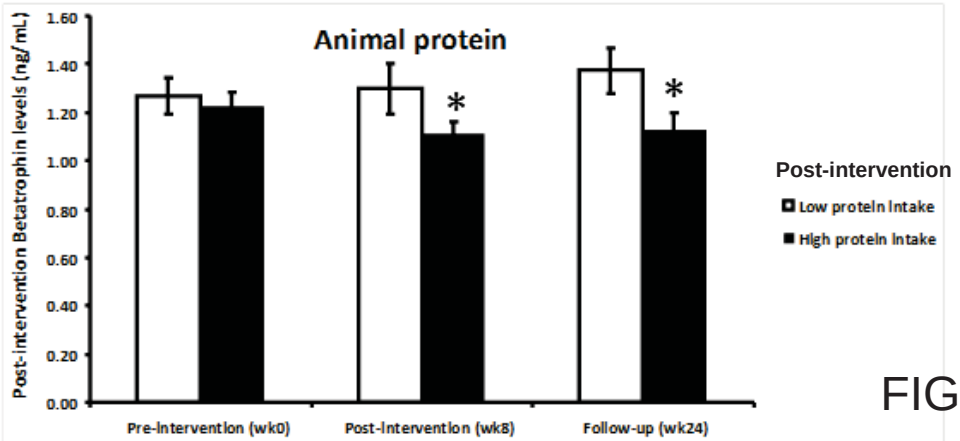

FIGURE 3

Table 3. Independent effects of dietary-induced changes (Δ=wk8-wk0) in body composition, biochemical parameters and protein intake on circulating betatrophin levels after nutritional intervention (post-intervention)

Model 1	**Standardized coefficients B**	**95%CI**		**P-value**
Δ Fat mass (kg)	0.419	0.025	0.093	0.001
Δ Fat-free mass (kg)	0.227	-0.006	0.091	0.082
Δ Fasting LDL_c (mg/dL)	-0.313	-0.007	-0.001	0.005
Δ Fasting HDL_c (mg/dL)	0.146	-0.005	0.025	0.173
Δ Fasting glucose (mg/dL)	-0.153	-0.005	0.001	0.150
Δ Fasting insulin (U/mL)	-0.060	-0.021	0.012	0.562
Corrected R^2 = 0.12				*0.008*
Model 2				
Δ Fat mass (kg)	0.440	0.030	0.102	0.000
Δ Fat-free mass (kg)	0.207	-0.009	0.090	0.107
Δ Fasting LDL_c (mg/dL)	-0.330	-0.008	-0.002	0.004
Δ Fasting HDL_c (mg/dL)	0.217	-0.001	0.031	0.058
Δ Fasting glucose (mg/dL)	-0.129	-0.005	0.001	0.233
Δ Fasting insulin (U/mL)	-0.063	-0.022	0.012	0.549
Post-intervention total protein intake (g/d)	-0.203	-0.011	0.000	0.060
Corrected R^2 = 0.16				*0.004*
Model 3				
Δ Fat mass (kg)	0.481	0.037	0.109	0.000
Δ Fat-free mass (kg)	0.236	-0.003	0.096	0.066
Δ Fasting LDL_c (mg/dL)	-0.351	-0.008	-0.002	0.002
Δ Fasting HDL_c (mg/dL)	0.221	0.000	0.031	0.046
Δ Fasting glucose (mg/dL)	-0.114	-0.005	0.001	0.286
Δ Fasting insulin (U/mL)	-0.079	-0.023	0.010	0.444
Post-intervention animal protein intake (g/d)	-0.267	-0.013	-0.002	0.012
Corrected R^2 = 0.20				*0.001*